

IN THE CLAIMS

Please cancel claims 14-24 and 29, amend claims 1, 4, and 5, and add new claims 31-38 as indicated below. The pending claims are as follows:

1. (Currently Amended) A modified thermostable DNA polymerase having a 3'-5' exonuclease activity, wherein in the $DX_1EX_2X_3X_4H$ sequence (D: aspartic acid, E: glutamic acid, H: histidine, X_1 , X_2 , X_3 and X_4 : any amino acid) ~~consisting of DX_1E sequence within the EXO exonuclease I region and a four amino acid length peptide adjacent to said glutamic acid(E) of the thermostable DNA polymerase having 3'-5' exonuclease activity~~, histidine(H) has been replaced by another amino acid.

2. (Original) The modified thermostable DNA polymerase according to claim 1, wherein in the $DX_1EX_2X_3X_4H$ sequence, histidine(H) has been replaced by an amino acid selected from the group consisting of aspartic acid, glutamic acid, tyrosine, alanine, lysine and arginine.

B⁴ 3. (Original) The modified thermostable DNA polymerase according to claim 1 having the following physicochemical properties:

- (1) DNA extension rate: at least 20 bases/second; and
- (2) thermostability: it is capable of retaining 10% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours.

4. (Currently Amended) The modified thermostable DNA polymerase according to claim 3 having the following physicochemical properties:

- (1) DNA extension rate: at least 30 bases/second;
- (2) thermostability: it is capable of retaining 40% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours; and

(3) amino acid sequence: in the ~~DIETLYH~~ sequence (~~D: aspartic acid, I: isoleucine, E: glutamic acid, T: threonine, L: leucine, Y: tyrosine, H: histidine~~) $DX_1EX_2X_3X_4H$ sequence (D: aspartic acid, X_1 : isoleucine, E: glutamic acid, X_2 : threonine, X_3 : leucine, X_4 : tyrosine, H:

histidine) located at the 141- to 147-positions in the amino acid sequence of SEQ ID NO: 2, histidine(H) has been replaced by another amino acid.

5. (Currently Amended) The modified thermostable DNA polymerase according to claim 4 having the following physicochemical properties:

(1) DNA extension rate: at least 30 bases/second;

(2) thermostability: it is capable of retaining 60% or more DNA polymerase activity of untreated DNA polymerase at pH 8.8 (determined at 25°C) after treatment at 95°C for 6 hours; and

(3) amino acid sequence: in the ~~DIETLYH~~ sequence (~~D: aspartic acid, I: isoleucine, E: glutamic acid, T: threonine, L: leucine, Y: tyrosine, H: histidine~~) DX, EX, X₂, X₃, X₄, H sequence (D: aspartic acid, X₁: isoleucine, E: glutamic acid, X₂: threonine, X₃: leucine, X₄: tyrosine, H: histidine) located at the 141- to 147-positions in the amino acid sequence of SEQ ID NO: 2, histidine(H) has been replaced by another amino acid.

34
6. (Original) The modified thermostable DNA polymerase according to claim 5, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by an amino acid selected from the group consisting of aspartic acid, glutamic acid, tyrosine, alanine, lysine and arginine.

7. (Original) The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by aspartic acid.

8. (Original) The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by glutamic acid.

9. (Original) The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by tyrosine.

10. (Original) The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by alanine.

11. (Original) The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by lysine.

12. (Original) The modified thermostable DNA polymerase according to claim 6, wherein in the amino acid sequence of SEQ ID NO: 2, histidine(H) at the 147-position has been replaced by arginine.

74
13. (Withdrawn) A gene encoding a modified thermostable DNA polymerase wherein in the DX₁EX₂X₃X₄H sequence (D: aspartic acid, E: glutamic acid, H: histidine, X₁, X₂, X₃ and X₄: any amino acid) consisting of DX₁E sequence within the EXO I region and four amino acid length peptide adjacent to said glutamic acid(E) of thermostable DNA polymerase having 3'-5' exonuclease activity, histidine(H) has been replaced by another amino acid.

14-24. (Canceled)

Sub C1
25. (Original) A reagent kit for amplifying nucleic acid, which comprises 2 kinds of primers, each of the primers being complementary to a DNA extension product of the other primer; dNTP; the thermostable DNA polymerase of any one of claims 1-12; divalent ion(s); monovalent ion(s); and a buffer solution.

26. (Previously Amended) A reagent kit for amplifying nucleic acid, which comprises 2 kinds of primers, each of the primers being complementary to a DNA extension product of the other primer; dNTP; the thermostable DNA polymerase of claim 1; magnesium ion; at least one of monovalent ions selected from the group consisting of ammonium ion and potassium ion; BSA (bovine serum albumin); a nonionic surfactant and a buffer solution.

27. (Previously Amended) A reagent kit for amplifying nucleic acid, which comprises 2 kinds of primers, each of the primers being complementary to a DNA extension product of the other primer; dNTP; the thermostable DNA polymerase of claim 1; magnesium ion; at least one of monovalent ions selected from the group consisting of ammonium ion and potassium ion; BSA (bovine serum albumin); a nonionic surfactant; a buffer solution and an antibody capable of suppressing at least one activity selected from polymerase activity and 3'-5' exonuclease activity of the thermostable DNA polymerase.

B4 28. (Previously Amended) A DNA polymerase composition which comprises one or more kinds of modified thermostable DNA polymerases defined in claim 1.

29. (Canceled)

30. (Previously Amended) A reagent kit for producing a mutated DNA which comprises mutagenesis primers, dNTP and the thermostable DNA polymerase of claim 1.

31. (New) A method for improving amplification efficiency and/or fidelity comprising replacing histidine residue in the $DX_1EX_2X_3X_4H$ sequence (D: aspartic acid, E: glutamic acid, H: histidine, X_1 , X_2 , X_3 and X_4 : any amino acid) within the exonuclease I region of the thermostable DNA polymerase is replaced by another amino acid.

32. (New) A modified thermostable DNA polymerase according to claim 1 wherein said DNA polymerase is a-like DNA polymerase.

33. (New) A modified thermostable DNA polymerase according to claim 1 wherein said DNA polymerase is thermostable DNA polymerase.

34. (New) A modified thermostable DNA polymerase according to claim 1 wherein said DNA polymerase is selected from KOD DNA polymerase derived from Pyrococcus kodakaraensis KOD1, thermostable DNA polymerase derived from Pyrococcus furiosus and thermostable DNA polymerase derived from Thermococcus litoralis.

35. (New) A modified thermostable DNA polymerase according to claim 1 wherein said the DX₁EX₂X₃X₄H sequence is selected from DIETLYH or DIETFYH.

36. (New) A modified thermostable DNA polymerase according to claim 1 wherein histidine (H) has been replaced by an acidic amino acid to obtain the modified thermostable DNA polymerase having significantly reduced 3'-5' exonuclease activity as compared with the enzyme before modification.

37. (New) A modified thermostable DNA polymerase according to claim 1 wherein histidine (H) has been replaced by a neutral amino acid to obtain a modified thermostable DNA polymerase having improved amplifying efficiency.

38. (New) A modified thermostable DNA polymerase according to claim 1 wherein histidine (H) has been replaced by a basic amino acid to obtain a modified thermostable DNA polymerase having significantly improved 3'-5' exonuclease activity and/or fidelity on a DNA replication or amplification.
